

PERSPECTIVE

Thinking Differently about Cancer Treatment Regimens Jeff Settleman¹, João M. Fernandes Neto², and René Bernards²

Summary: Most experimental cancer drugs ultimately fail during the course of clinical development, contributing to the high cost of the few that are granted regulatory approval. Moreover, approved drugs often deliver only modest clinical benefit to patients with advanced disease due to the development of resistance. Here, we discuss opportunities we consider promising to overcome drug resistance associated with interactions between signaling pathways and the presence of multiple coexisting cell states within tumors with distinct vulnerabilities. We highlight how understanding drug-resistance mechanisms can enable innovative treatment regimens that deliver longer-lasting benefit to patients.

The adage “If you do what you did, you get what you got” is often used to remind us that meaningful change sometimes requires a fundamentally different approach. This certainly appears to apply to cancer drug development. The vast majority of novel cancer drugs are developed as single-agent therapies and are delivered to patients at a maximum tolerated dose. For a variety of reasons, both economic and regulatory, this drug development model has remained virtually unchanged for several decades, resulting in the attrition of more than 30% of early-stage investigational agents due to lack of single-agent efficacy (1). However, a drug without single-agent activity is not necessarily a bad drug. As one example, small-molecule inhibitors of the *BRAF*^{V600E} oncoprotein do not elicit clinical responses in most patients with *BRAF*-mutant colorectal cancer (a case of intrinsic resistance; ref. 2), but do provide benefit to most patients with *BRAF*-mutant melanoma (3). It is fortunate in retrospect that Tsai and colleagues (4) developed the *BRAF* inhibitor PLX4720 in melanoma, even though Davies and colleagues (5) had initially found that a significant fraction of colon cancers similarly harbor activating mutations in the *BRAF* gene. Otherwise, the drug known as vemurafenib, which has become a major clinical and commercial success, would most likely also have ended up in the graveyard of compounds lacking single-agent activity, together with many other potentially useful candidate drugs (4). We discuss here a number of rational drug combination strategies that we feel have the potential to decrease attrition rates during clinical development and deliver additional benefit for patients with cancer, while highlighting some of the current challenges in the field.

¹Oncology R&D Group, Pfizer Worldwide Research and Development, San Diego, California. ²Division of Molecular Carcinogenesis and Oncode Institute, The Netherlands Cancer Institute, Amsterdam, the Netherlands.

Corresponding Author: René Bernards, Division of Molecular Carcinogenesis, The Netherlands Cancer Institute, Plesmanlaan 121, 1066 CX Amsterdam, the Netherlands. Phone: 31-20-512-6973; Fax: 31-205-121954; E-mail: r.bernards@nki.nl

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SYNTHETIC LETHAL DRUG COMBINATIONS

Based on drug approval history, it has been argued that drugs that lack single-agent activity are not worth pursuing in combination, due to low success rate and marginal combination benefit in the face of considerable combination toxicity (6). However, the 18 or so combination therapies these investigators evaluated largely consisted of combinations of targeted agents that lacked single-agent activity with chemotherapies or hormonal therapies, combinations for which there was no rational mechanistic basis. In many trial-and-error combination studies, each compound has demonstrated some single-agent activity, and the expectation is that the combination would be superior. One salient example that highlights the risk of this approach is the combination of cetuximab (EGFR inhibitor) and bevacizumab (VEGF inhibitor), two blockbuster drugs approved for the treatment of colon cancer. A large study in colon cancer found that the addition of cetuximab to a regimen of chemotherapy and bevacizumab resulted in worse outcome for some patients (7). These findings highlight the potential harm to patients of combination treatments without rational basis. The substantial number of trials testing combinations with PD-1 or PD-L1 antibodies also highlights the lack of mechanistic basis for many such trials.

In the case of *BRAF*-mutant colon cancer, genetic screens to identify synthetic lethal interactions, together with biochemical analyses, have revealed that *BRAF* inhibition results in feedback reactivation of EGFR. Moreover, a combination of *BRAF* and EGFR inhibitors was shown to be effective in preclinical models of *BRAF*-mutant colon cancer (8, 9). Based on a positive phase III study, this drug combination was recently approved for this indication (10). This example highlights how fundamental insights into cross-talk and feedback mechanisms that operate in cancer cells can help inform rational and effective combination therapies (Fig. 1A). This type of approach may therefore be useful to resuscitate compounds that were unsuccessful as single agents in oncology, such as inhibitors of the unfolded protein response kinase ERN1. Such drugs were shown to be potent inhibitors of the ERN1 kinase, but did not have significant effects on a large panel of cancer cell lines (11). Moreover, emerging evidence indicates that the highly

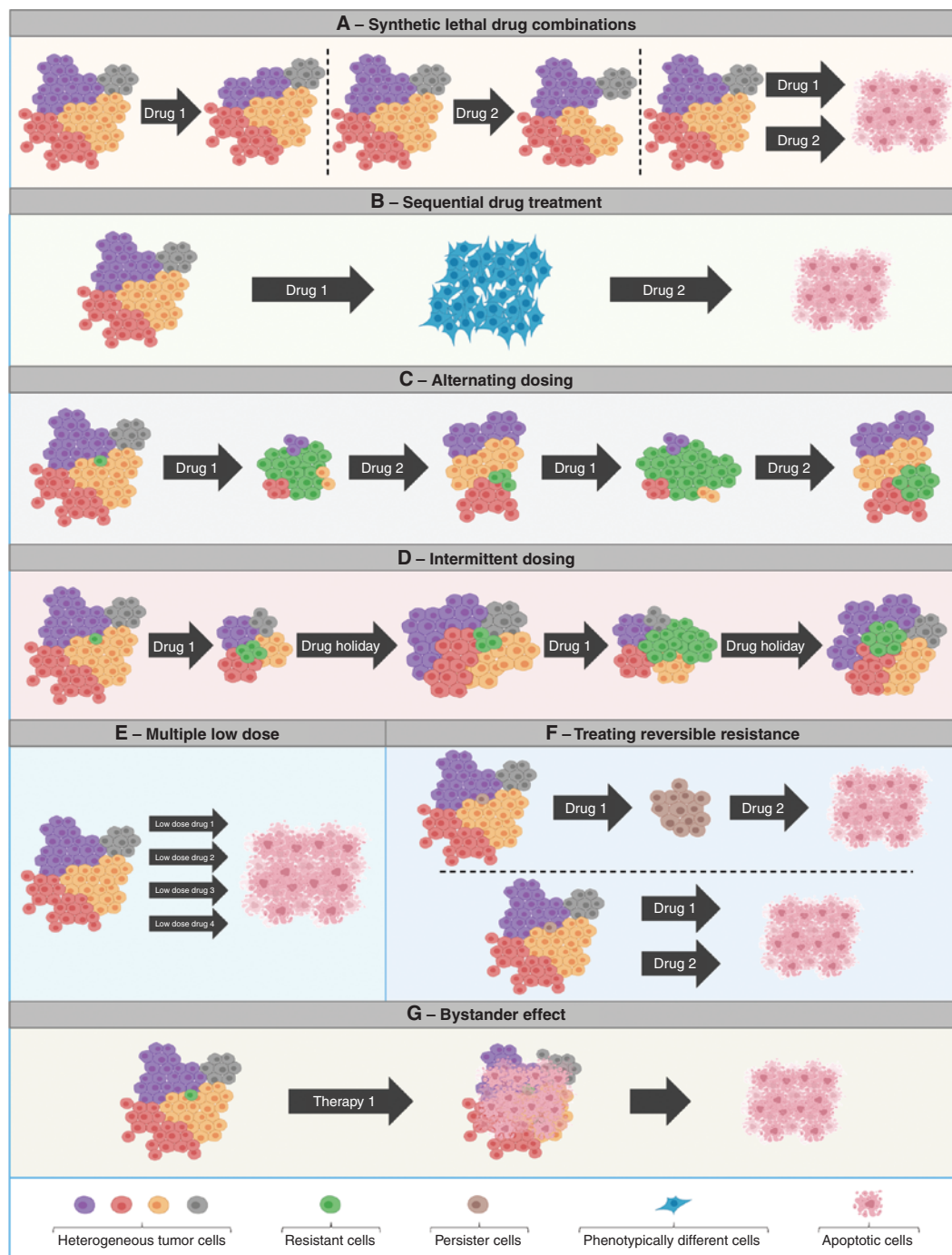


Figure 1. Schematic representation of the drug treatment regimens discussed. **A**, Synthetic lethal drug combinations. Synthetic lethality refers to a situation in which two agents are individually nonlethal, but become lethal when used in combination. **B**, Sequential drug treatment. The first drug is used to bring the cancer cells in a metastable state having an acquired vulnerability (e.g., senescence); the second drug acts on the acquired vulnerability (e.g., a senolytic agent). **C**, Alternating dosing. Drug resistance comes with an associated fitness cost. Resistance to drug 1 is associated with an acquired vulnerability to drug 2. Alternating treatment of the drug-sensitive and drug-resistant populations can keep the tumor in control over longer periods of time. **D**, Intermittent dosing. Termination of drug administration following development of resistance can result in tumor regression due to a disadvantage of the drug-resistant population in the absence of drug. After a drug holiday, the drug-resistant population is depleted and the drug-sensitive population tumors respond again to the original drug. **E**, Multiple low dose. Targeting the same oncogenic signaling pathway with multiple drugs, each at low dose, can add up to complete pathway inhibition, while at the same time reducing the pressure on each of the nodes to develop resistance mutations. **F**, Treating reversible resistance. A minor fraction of a cancer may be epigenetically distinct, such that these cells enter a state of dormancy during treatment, which would allow repopulation of the tumor following termination of therapy. Targeting these “drug-tolerant persisters” with selective drugs can eliminate this population, preventing tumor regrowth. **G**, Bystander effect. A therapy that kills the majority of the cancer cells can lead to killing of drug-insensitive cells, for example by the release of signals (e.g., cytokines) by the dying cancer cells. Illustrations were created with BioRender.com.

selective KRAS^{G12C} inhibitors produce only modest clinical efficacy as single agents due to adaptive resistance and could benefit from treatment combinations to extend response duration (12–16). Indeed, CRISPRi modifier screens have identified “collateral dependencies” of cancer cells treated with KRAS^{G12C} inhibitors that inform potentially effective combinations with this drug (17). Collectively, these data point toward the utility of unbiased genetic approaches to identify rational combination therapies that may provide superior benefit as compared with the corresponding monotherapies.

SEQUENTIAL DRUG TREATMENT

A potential challenge associated with drug combinations identified through synthetic lethality genetic screens is the issue of combination toxicity in the clinic. This raises the question of whether it is possible to avoid combination toxicity by developing treatment regimens that show synergy without concomitant drug administration. Theoretically, this could be achieved if the first of two drugs induces a major vulnerability in the cancer cell that is targeted by a second drug to kill the cell (Fig. 1B). Indeed, Fang and colleagues (18) demonstrated that although a combination of PARP and WEE1 inhibitors was highly toxic in animal models of ovarian cancer, the sequential therapy with these two drugs caused minimal toxicity and showed significant efficacy. That this sequential therapy showed synergy is explained by the finding that monotherapy-induced DNA damage or G₂ cell-cycle arrest was maintained after drug removal, allowing the acquired vulnerability to be maintained during the second drug treatment cycle (18). Similarly, it has been shown that sequential, but not simultaneous, treatment of triple-negative breast cancer cells with EGFR inhibitors and DNA-damaging drugs leads to efficient cell killing (19). Finally, preclinical data also support the use of sequential drug regimens for combination immunotherapies (reviewed in ref. 20).

Successful sequential drug treatment strategies require that a metastable state is induced by the first therapy that persists beyond cessation of the first therapy and is associated with a significant new vulnerability. We and others have argued that induction of senescence in cancer cells might be an effective first step in a sequential drug treatment strategy, as senescence is a stable proliferation arrest characterized by major changes in metabolism, gene expression, and cytokine production (21, 22). Such senescence-induced cellular changes might cause vulnerabilities that enable selective killing of senescent cells with drugs that specifically target them—so-called senolytic drugs (Fig. 1B). Therapy-induced senescence has been described as a side effect of several cancer therapeutics, including many chemotherapies (23). Thus, cancer cells are still able to become senescent, even though they have successfully bypassed the classic telomere-shortening senescence checkpoint (24). Indeed, both chemical and genetic screens have identified targets and compounds to induce senescence in cancer or kill senescent cancer cells (25–27). Moreover, preclinical evidence indicates that a combination of a pro-senescence drug and a senolytic compound may be effective therapeutically (28, 29).

Senescent cells also secrete a number of cytokines, due to the activation of the cGAS–STING pathway as a result of the accumulation of cytoplasmic nucleic acids in senescent cells (30). This attracts a broad spectrum of inflammatory cells, including T cells, NK cells, and macrophages to the senescent cancer cell mass, which provides additional opportunities to eliminate senescent cancer cells. For instance, it was shown that pancreatic cancer cells rendered senescent by a combination of a CDK4/6 inhibitor and a MEK kinase inhibitor are efficiently cleared by treatment with PD-1 checkpoint immunotherapy (31).

The notion that pretreatment with a drug can sensitize to PD-1 therapy has also been observed clinically. Induction therapy of patients with metastatic breast cancer with some, but not all, forms of chemotherapy resulted in enhanced responses to nivolumab (anti-PD-1). Pretreatment with cisplatin and doxorubicin in particular was associated with inflammatory gene signatures and response to PD-1 therapy (32). The stimulatory effect of certain chemotherapies on the PD-1 response may be caused by immunogenic cell death or by induction of senescence, leading to changes in the tumor microenvironment due to cytokine production (33, 34). These examples highlight the considerable promise of sequential treatments for future clinical application.

ALTERNATING DOSING

It is observed generally in the clinic that second-line therapies are less effective than first-line therapies, with third-line being even less effective than second-line treatments. However, it has been appreciated for nearly 60 years that resistance to one cancer drug might come at a “fitness cost,” which can yield a vulnerability to another drug, a phenomenon referred to as “collateral sensitivity” (35). Identification of collateral sensitivities of drug-resistant cancer cells represents a large untapped opportunity for the discovery of potentially important drug targets for selectively killing drug-resistant cancer cells. We have collectively been remarkably unsuccessful in finding collateral sensitivities of chemotherapy-resistant cancer cells, most likely due to the heterogeneity in chemotherapy-resistance mechanisms. The recent identification of sensitization to checkpoint immunotherapy by some chemotherapies discussed above is a notable exception. However, there is reason to be more optimistic that collateral sensitivities associated with resistance to targeted cancer drugs may be more homogeneous. This optimism is based on the limited options cancer cells have to develop resistance to targeted cancer drugs. Most often, such drug-resistant cancer cells will reactivate the inhibited pathway through upstream, downstream, or parallel pathway activation, which makes the acquired vulnerabilities more predictable. As one example, biochemical studies have demonstrated that resistance of melanoma to BRAF inhibitors is associated with a marked increase in sensitivity to histone deacetylase (HDAC) inhibitors. A pilot study in patients demonstrated that a brief treatment of patients with BRAF inhibitor-resistant melanoma with HDAC inhibitors eliminated the drug-resistant cell population (36). This model suggests a treatment strategy in

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which the drug-sensitive and drug-resistant subpopulation is targeted in an alternating fashion (Fig. 1C). This alternating treatment concept is currently being tested in a phase I trial (NCT02836548). Along the same lines, it has been observed that resistance of *EGFR*-mutant lung cancer cells to selective *EGFR* inhibitors is associated with a greatly increased sensitivity to AURORA kinase inhibitors, making AURORA kinase inhibition a collateral sensitivity of *EGFR* inhibitor-resistant lung cancers (37, 38). The use of AURORA kinase inhibitors in patients having *EGFR* inhibitor-resistant NSCLC is also undergoing clinical testing (NCT04085315).

INTERMITTENT DOSING

A relatively rare clinical phenomenon is the observation that termination of drug administration following development of resistance can lead to tumor regression. Moreover, after such a “drug holiday,” tumors often have regained sensitivity to the original drug—the so-called “retreatment response” (ref. 39; Fig. 1D). This phenomenon is most readily explained by an addiction of the drug-resistant cell to the drug, leading to a selective disadvantage of the drug-resistant population in the absence of drug. Indeed, in preclinical models of melanoma, intermittent dosing with BRAF inhibitors provides longer-lasting tumor control as compared with continuous dosing (40). However, recent clinical data appear to indicate that intermittent BRAF inhibitor dosing is inferior to continuous drug administration, highlighting the challenge of translating dosing and treatment schedules from mice to humans (41).

MULTIPLE LOW-DOSE TREATMENT

In advanced cancers, development of resistance is almost unavoidable due to secondary mutations. When targeted drugs are used as single agents, resistance mutations often emerge that involve mutations in the drug target itself. For instance, secondary mutations in the genes encoding the *BCR-ABL*, *EGFR*, and *ALK* kinases have been described upon inhibition of these kinases, but mutations in genes that act either upstream, downstream, or in parallel to the oncogenic pathway that is being targeted have been observed as well (42). To avoid this type of resistance, “vertical targeting” of two components in the same oncogenic signaling pathway has been shown to lead to longer-lasting therapeutic responses. Thus, in *BRAF*-mutant melanoma and lung cancer, combined inhibition of the BRAF and MEK kinases was more effective than treatment with the single agents (43, 44). In preclinical models of *BRAF*-mutant melanoma, it was possible to forestall resistance through a triple combination of BRAF, MEK, and ERK inhibitors (each at high concentration) to prevent resistance, but drugs had to be administered intermittently to limit toxicity (45).

It is generally believed that for a drug combination to be efficient, each drug must have considerable single-agent activity to suppress clonal diversity. This is seen, for example, in the treatment of HIV, where combinations of drugs that target the viral reverse transcriptase, protease, and integrase proteins are used to prevent resistance (46). However, recent evidence indicates that vertical targeting of *EGFR*-mutant lung cancers

with three or four drugs that act in the *EGFR* signaling pathway can be an effective treatment strategy, even when the four drugs are used at only 20% of the effective single-agent concentration (47). In a related study, low-dose inhibition of RAF and ERK proved effective in *KRAS*-mutant cancers (48). A key difference between the viral and cancer therapies is that in the latter, all drugs target the same signaling pathway, thereby allowing synergistic inhibition at low drug concentrations. At the same time, partial inhibition of multiple nodes of a pathway reduces the selective pressure on these nodes to gain a resistance mutation. These observations of synergy between low drug doses in the MAP kinase pathway are clearly at odds with the generally held view that this pathway serves to amplify signals. As such, these data highlight that much remains to be learned about cross-talk and feedback mechanisms that operate in this signaling context. Collectively, these findings emphasize that we may have to consider combinations of more than two targeted agents, preferably in the same signaling cascade, to forestall resistance (Fig. 1E). That such combinations are not necessarily overly toxic was demonstrated in a recent study, in which BRAF, MEK, and *EGFR* inhibitors were successfully combined in the clinic (10).

TREATING REVERSIBLE RESISTANCE

Much of our current understanding of cancer drug resistance mechanisms has been informed by the identification of specific mutational events that underlie stable, propagatable states of resistance (49). The development of such resistance-conferring mutations is certainly consistent with fundamental principles of Darwinian evolution, and likely reflects the stochastic emergence of such mutations at low frequency in tumor cell populations prior to drug exposure—resulting in the outgrowth of stably drug-resistant cancer cell clones through natural selection. However, nonmutational and therefore potentially reversible mechanisms of drug resistance are becoming increasingly recognized. For example, the drug-induced senescence described above may be reversible, such that, upon drug withdrawal, such “senescent” cancer cells could lose their senescence features and resume proliferation. Similarly, cancer cell “dormancy” is another relatively poorly understood cell state associated with transient quiescence and treatment resistance (50).

The differentiation and dedifferentiation of cancer cells has also been linked to fluctuation between states of differing drug sensitivity and resistance. For example, the epithelial-mesenchymal transformation (EMT; ref. 51), a slowly reversible state change, has been associated with the acquisition of drug resistance in many epithelial cancers (52). Moreover, the molecular features exhibited by the generally more treatment-refractory mesenchymal cell state are often shared by cancer stem cells, a subpopulation of phenotypically “plastic” tumor cells that have also been associated with drug resistance (53).

Another form of reversible drug resistance has been described as the “drug-tolerant persister” (DTP) state (54). When propagated in the laboratory, clonal cancer cell lines have been found to exhibit phenotypic heterogeneity that typically includes the presence of small subpopulations of cells that do not share the same vulnerabilities to drug treatment as are seen with the bulk population of cells (54).

Consequently, these DTPs survive an otherwise lethal drug exposure and maintain viability for long periods of time in the presence of continuous drug exposure. Significantly, upon drug withdrawal, DTPs can resume proliferation, giving rise to a largely drug-sensitive cell population. Collectively, reversibly senescent cells, dormant cells, mesenchymal cells, cancer stem cells, and DTPs share features that suggest that they are highly related and may in fact reflect common underlying mechanisms. Consequently, they may exhibit overlapping vulnerabilities that could provide opportunities to target such tumor cell populations with therapeutics aimed specifically at overcoming drug resistance as part of a combination treatment regimen that also targets the bulk cell population (Fig. 1F).

The reversible nature of these various states of drug resistance implicates epigenetic regulation. Indeed, the role of epigenetic control in the EMT process in cancer cells is now well established (55). Similarly, epigenetic changes appear to be the critical determinants of “decisions” to enter and exit stem cell states—including those exhibited by cancer stem cells (56). Senescent cells are characterized by a distinct organizational structure of heterochromatin, suggesting that epigenetic regulation is likely to play a role in the transition into and out of senescence (57). DTPs have also been found to harbor distinct chromatin features, including a repressed chromatin state associated with specific alterations in histone methylation (54, 58). Importantly, despite their resistance to “conventional” therapeutics, these distinct chromatin features associated with reversibly drug-tolerant states appear to yield specific therapeutic vulnerabilities. For example, the DTP state can be disrupted by targeting the KDM5 family histone demethylases, the SETDB1, G9a, and EZH2 methyltransferases, and the class I histone deacetylases (54, 58, 59).

In addition to chromatin regulators, various other vulnerabilities have been associated with reversible states of drug resistance. These include, for example, the senolytic agents described above, cancer stem cell-targeted agents such as BMI1 inhibitors (60) and the antibiotic salinomycin (61), as well as agents that have been reported to selectively kill mesenchymal cells, such as the multikinase inhibitor dasatinib (62). In addition to epigenetic modulators, other potential target-associated vulnerabilities have been described for DTPs, including the cancer stem cell-enriched protein ALDH1A1 (63) and the selenocysteine family protein GPX4 (64). Recently, it was reported that targeting YAP-TEAD pathway signaling disrupts a senescence-like state of cancer cell dormancy associated with resistance to EGFR kinase inhibition, with the potential to prolong treatment effects (65).

Considering that these nonmutational mechanisms of drug resistance reflect a form of dynamic phenotypic heterogeneity that appears to be broadly present within tumor cell populations, the potential benefit resulting from disruption of such states could be substantial. Thus, combination treatments could be deployed in which an agent that targets the bulk population of cancer cells could be combined with an agent that selectively targets the “preexisting” phenotypically distinct and more treatment-refractory subpopulation of cells, with the goal of forestalling resistance (Fig. 1F). An alternative approach would be to target mechanisms that enable the transition of cancer cells into the transiently

maintained state of drug tolerance—for example, with agents that block the EMT process. Similarly, it may be possible to drive the drug-resistant subpopulation of cells into a state that “matches” the drug sensitivity of the bulk population—effectively “leveling the playing field” by promoting a greater degree of homogeneity among tumor cell subpopulations. In any of these treatment schemes, the “up-front” administration of the combination therapy is expected to delay or prevent the emergence of drug resistance. However, it is also possible that such treatments could be sequenced, especially if the transition between plastic states of sensitivity and resistance requires significant time, thereby yielding a window during which treatments could be spaced (Fig. 1F). If effective, this approach provides an opportunity to reduce the potential for dosing limitations imposed by overlapping toxicities between the combination agents.

OVERCOMING HETEROGENEITY

The presence of intratumor heterogeneity represents a formidable obstacle to successful therapy, independent of the treatment strategy and cancer type. Changes in selective pressures during the lifetime of a cancer can yield a diversity of subclones having different genotypes. Nevertheless, there are reasons to be hopeful that heterogeneity can ultimately be overcome. First, cancer subclones share “truncal” mutations that can be targeted for therapy (66). For instance, the anti-HER2 antibody trastuzumab is very effective both in HER2-positive metastatic breast cancer and as an adjuvant therapy for early breast cancer (67). Second, sequencing of tumor subclones provides clear evidence for convergent evolution, indicating that subclones have limited options to evolve (66). Third, even patients with advanced disease (and consequently likely to have more heterogeneous tumors) can still have long-lasting responses, indicating that in these patients, drug-resistant subclones were unable to dominate the tumor cell population. In the treatment of advanced melanoma, secondary resistance mutations to BRAF inhibitor therapy occur so frequently that survival benefit is limited (3). In contrast, treatment of such patients with immunotherapy leads to a subgroup of patients experiencing very long survival (68). This is not because mutations that confer resistance to immunotherapy cannot occur, as sequencing of resistant tumors has shown (69). Hence, it seems plausible that the superior responses of immunotherapies compared with MAP kinase inhibitors in melanoma can be explained by the killing of resistant cancer cells through a bystander effect. Such bystander effects can be caused by signals (e.g., cytokines) that are secreted by the dying cancer cells. Although the precise mechanism of action of bystander effects is understood only poorly, this clearly represents an opportunity to overcome intratumor heterogeneity (Fig. 1G).

CONCLUSIONS

First, the paradigm that new oncology drugs should first show single-agent activity before combinations are considered needs to be revisited. Rather than waiting for a phase II (single-agent) trial to report results, drug developers could invest earlier in finding the best combinations and consider

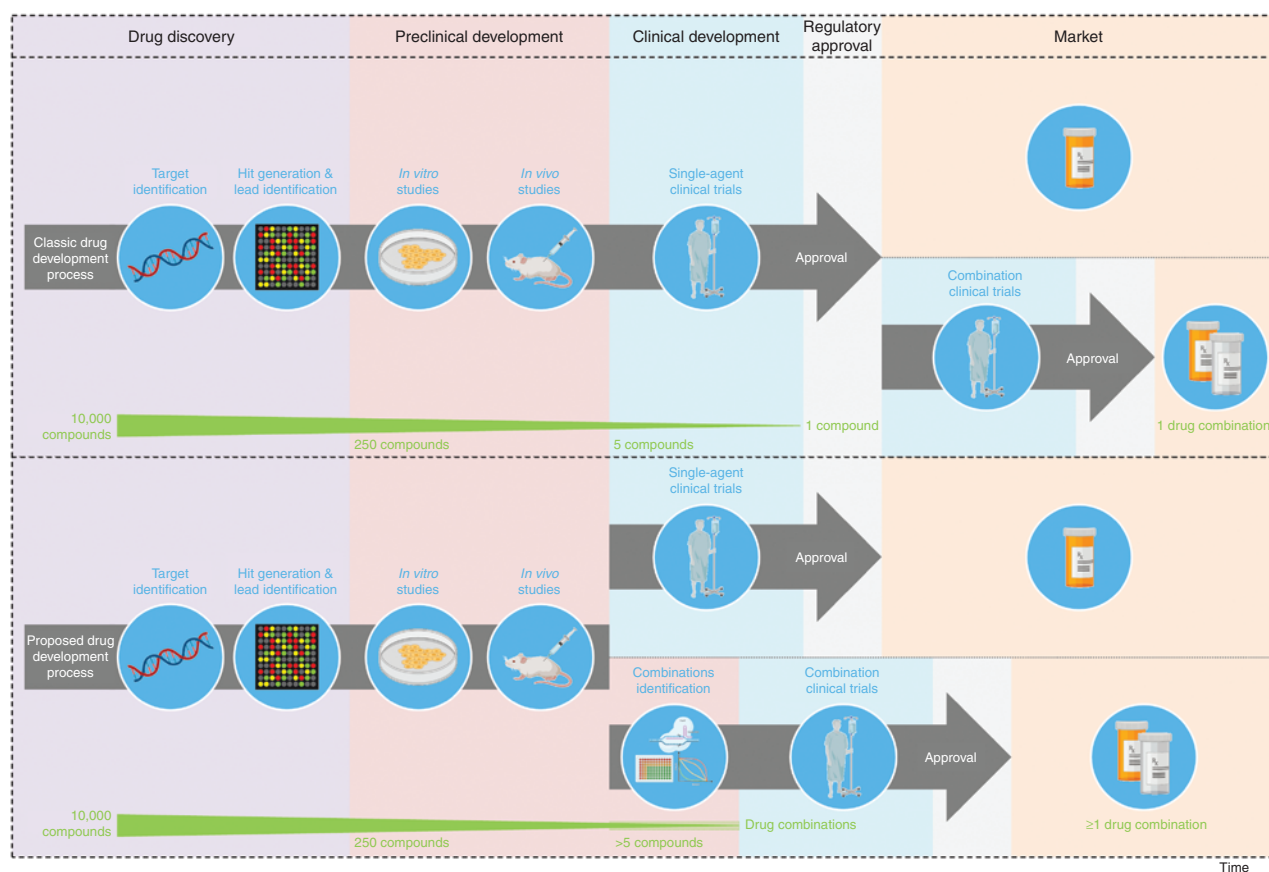


Figure 2. Schematic representation of the drug-development process. Top, in conventional drug development, new chemical entities are first tested as single agents in clinical studies. Only after single-agent approval, combination studies are considered. Bottom, proposed drug development model. During the preclinical phase of drug development, genetic and biochemical studies are performed to identify rational and effective combination therapies. These combination regimens are developed in parallel to (or even instead of) the development of the novel chemical entity as a single agent. This model could lead to less attrition of new chemical entities and longer-lasting therapeutic benefits for patients. Illustrations were created with BioRender.com.

developing the drugs further in combination after phase I (Fig. 2). A good example of this is the clinical development of SHP2 inhibitors. Although they may have single-agent activity in tyrosine kinase-driven cancers (70), ample pre-clinical evidence indicates that these drugs are more effective when combined with MEK inhibitors (71–73). Although the dose escalation of one of these agents (RMC4630) as single agent is still ongoing, studies are also already under way to test this inhibitor in combination with MEK inhibitors (NCT03989115). Fortunately, the FDA appreciates the need for codevelopment of such drug combinations and has recently released a guidance document to assist in the development of “two or more new drugs that have not been previously developed for any indication to be used in combination to treat a disease or condition” (<https://www.fda.gov/media/80100/download>).

Second, more efforts could be directed to identifying the vulnerabilities of drug-resistant cancer cells. Identification of such vulnerabilities may result in development of second-line therapies that are potentially more effective than the first-line therapy—rather than less effective, as is currently often the case. The study of such vulnerabilities can also uncover new classes of drug targets that are distinct from the oncogenic

drivers that are currently a main focus of drug development. Alternating dosing schedules that target the drug-sensitive and drug-resistant populations in a periodic fashion may then be used to control the tumor over prolonged periods of time.

Finally, greater effort could be made to study the synergistic effects of multiple targeted agents used in combination. The notion that such drugs can still be combined efficiently when used at low dose may provide ample opportunity to combine such agents with limited toxicity.

Authors' Disclosures

J. Settleman reports personal fees from Pfizer Inc. during the conduct of the study. R. Bernards reports being a shareholder of Oncosec BV during the conduct of the study. No disclosures were reported by the other author.

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Jeff Settleman, João M. Fernandes Neto and René Bernards

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